

AD-A034 281

MONTANA UNIV MISSOULA DEPT OF MICROBIOLOGY  
SEROLOGICAL DIAGNOSIS OF GONORRHEA.(U)  
DEC 76 J A RUDBACH

F/6 6/5

N00014-74-A-0013-0001  
NL

UNCLASSIFIED

1 OF 1  
AD  
A034281

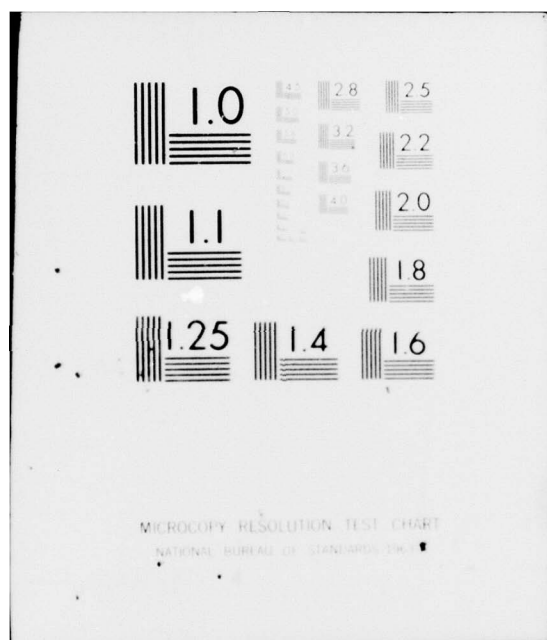


END

DATE  
FILMED

2-77





ADA034281

12

OFFICE OF NAVAL RESEARCH

Contract N00014-74-A-0013-0001 and -0002

Task No. NR 136-958

and

Contract N00014-76-C-0268

Task No. NR 204-004

FINAL REPORT

Serological Diagnosis of Gonorrhea

by

Jon A. Rudbach

Department of Microbiology  
University of Montana  
Missoula, Montana 59812

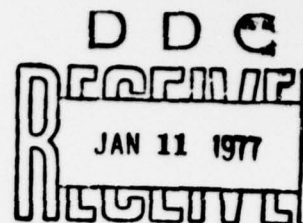
1 December 1976

Reproduction in whole or in part is permitted for  
any purpose of the United States Government

This document has been approved for public release;  
its distribution is unlimited.

4 DDC  
RECEIVED  
JAN 11 1977  
D

(See form 1473)



a) Summary of research accomplished.

An antigen was extracted from T-1 phase of Neisseria gonorrhoeae strain F62 which was grown in a medium containing  $^{14}\text{C}$ -glucose. This antigen was extracted with sodium deoxycholate and was purified partially by fractional ethanolic precipitation and centrifugation (1,2). This  $^{14}\text{C}$ -labeled antigen was used in an attempt to develop a radioimmunoprecipitation assay which could detect antibodies in human sera specific for N. gonorrhoeae. The  $^{14}\text{C}$ -antigen was incubated with samples of sera from patients with gonorrhea and the radioactive antigen which had bound to antibodies was precipitated as an immune complex by rabbit antiserum to human immunoglobulins (2).

This procedure appeared to offer promise for detecting antibodies to N. gonorrhoeae in human sera. In general, it was found that acutely infected (longer than 7 days) patient's sera and convalescent (cured less than 6 months before) patient's sera reacted more strongly with the gonococcal antigen than did control sera from presumably uninfected individuals (3,4). Sera from male gonococcal infected patients could be assigned correctly as positive by the radioimmunoassay (RIA) procedure 93% of the time. Well documented male negative control sera were called negative in radioimmunoassays in 75% of the cases. This gave a total correct diagnosis rate of negative and positive males by RIA of 90%. Sera from females was correctly assigned as positive or negative in 89% of the samples tested. The combined correct diagnosis rate for sera from males and females was 90% (4).

It was found that female negative control sera demonstrated a lower percentage of false positive reactions than did sera of males. Also, sera from females had a lower background reactivity with the  $^{14}\text{C}$ -labeled gonococcal antigen than did sera from control male subjects (4).

SECTION	White Section	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	Ref Section			
INDICATED				
TIFICATION				
ISTRIBUTION/AVAILABILITY CODES				
SIGL.	AVAIL.	NOG/W	SPECIAL	
				A

The chemical, physical, and biological properties of the gonococcal specific antigen were studied (4,5). It was found that the specific antigenic activity was destroyed by boiling for 5 min, by digestion with proteolytic enzymes, and by periodate oxidation. Antigenic activity was not destroyed by heating at 56 C for 1 hr, by treatment with 0.1 N acid or alkali, by saponification, or by digestion with ribonuclease or deoxyribonuclease. It was found that the specific antigen was not contained in T3 phase gonococci or in an endotoxin preparation of T1 phase gonococci. A fraction containing specific antigenic activity was eluted in the void volume from Sephadex G-200. Fractions containing specific antigenic activity were found in the regions of 40% and 10% sucrose following ultracentrifugation in a sucrose gradient. A model for the structure of the gonococcal specific antigen was proposed to account for the above findings. It was hypothesised that the gonococcal specific antigen was a low molecular weight, linear or loosely folded glycoprotein. Large, apparently stable, aggregates of the antigen appeared to occur spontaneously (5).

Extracts of the gonococci were prepared as described previously, except that non-radioactive glucose was used in the growth medium. Preparations of these gonococcal antigens could be labeled with  $^{125}\text{I}$  by a very mild diffusion method (2-5). It was found that when these externally labeled gonococcal antigen preparations were used in the radioimmunoprecipitation assay procedure to detect gonococcal antibodies in human sera, the results shown in Table 1 were obtained. Sera from culturally positive males gave positive reactions in the RIA in 79% of the cases; well documented negative male sera reacted negatively in 21 out of 32 samples (66% correct). Sera from gonococcal positive females reacted positively in 81% of the samples tested, and negative



female sera reacted negatively 73% of the time. This gave an overall diagnosis rate with the  $^{125}\text{I}$ -RIA of 77% correct, 13% incorrect, and 10% indecisive.

In an effort to answer the criticism that the serum component reacting with gonococcal antigen might have been C-reactive protein and not specific antibody (5), the following experiments were performed. The RIA reactivities of selected sera were compared with the reactivity of these sera in a latex-agglutination test for C-reactive protein (CRP). However, no correlation was observed between the RIA reactivity and CRP reactivity of 62 sera tested. Therefore, CRP was not being detected in our assay (5).

A cooperative blind study, to determine the efficiency of the RIA on sera from cases of gonorrhea of short duration, was initiated with Dr. M. C. Shepard at Camp Lejeune, N.C. (1-3). Serum samples were collected from gonococcal infected individuals and from control (non-infected) individuals. The histories of the patients were sent to Dr. G. T. Strickland, NMRI, Bethesda, MD. The diagnosis of these 600 coded sera based on RIA results was as follows: 301 (50.2%) demonstrated positive reactivity; 207 (34.5%) were diagnosed as negative; and 92 (15.3%) gave indecisive RIA reactivity (5). The results of the assays on the individual sera were sent to Dr. Strickland for examination of correlation of the RIA with the patients' histories. Unfortunately, the results were not available in time to be included in this FINAL REPORT.

Several other phases of the investigation were in progress and were terminated when the contract expired. The nascent results which were just being obtained are summarized below:

Experimental methods were being worked out to examine the qualitative nature of the antibodies in human sera which reacted with gonococcal antigen in the RIA (5). Hopefully, this information would have been used to modify

the assay procedure so that an indication of the duration of the infection could have been gleaned from the test results. Also, it was hoped that vaginal and seminal secretions could have been tested for secretory antibodies to the gonococcus. However, none of these experiments had, as yet, yielded meaningful results.

On a limited scale we had initiated studies to determine the feasibility of differentiating cultures of virulent N. gonorrhoeae from related organisms by pyrolysis-gas-liquid-chromatography (5). Preliminary studies indicated that this technique could be used to determine whether or not certain gram negative diplococci, cultured from patient exudates, but which did not ferment glucose were virulent, but atypical gonococci or were atypical saprophytic Neisseria (or related genera).

Table 1. Summary interpretation of reactivity of sera with  $^{125}\text{I}$ -labeled gonococcal antigen in radioimmunoprecipitation assay.

Sex of patient	Patient history	Number tested	<u>RIA diagnosis</u> Diagnosis No.		Percentage correct
Male	positive	72	P <sup>a</sup>	57	79
			N <sup>b</sup>	7	
			I <sup>c</sup>	8	
	well doc. <sup>d</sup> negative	32	P	8	66
			N	21	
			I	3	
	ill doc. <sup>e</sup> negative	66	P	31	39
			N	26	
			I	9	
Female	positive	58	P	47	81
			N	6	
			I	5	
	well doc. negative	96	P	14	73
			N	70	
			I	12	
	ill doc. negative	5	P	0	80
			N	4	
			I	1	
Total of male and female	positive	130	P	104	80
			N	13	
			I	13	
	well doc. negative	128	P	22	71
			N	91	
			I	15	
	positive and well doc. neg. cumulative	258	correct	195	76
			incorr.	35	
			Indecis.	28	

<sup>a</sup>P-positive

<sup>b</sup>N-negative

<sup>c</sup>I-indecisive (within 2 standard deviations of the mean dividing positive and negative)

<sup>d</sup>Well doc.-well documented negatives (good culture methods and history)

<sup>e</sup>Ill doc.-ill documented negatives (no culture and poor history)



b) Index of technical reports.

1) Rudbach, J. A. STATUS REPORT No. 1, Serological diagnosis of gonorrhea, Contract N00014-74-A-0013-0001, Task No. NR 136-958, 22 February 1974.

2) Rudbach, J. A., M. K. Luoma, and W. R. Cross. ANNUAL REPORT NUMBER 1, Serological diagnosis of gonorrhea, Contract N00014-74-A-0013-0001, Task No. NR 136-958, 31 August 1974.

3) Rudbach, J. A. and M. K. Luoma. STATUS REPORT No. 2, Serological diagnosis of gonorrhea, Contract N00014-74-A-0013-0002, Task No. NR 136-958, 26 February 1975.

4) Rudbach, J. A., M. K. Luoma, and E. C. B. Milner. ANNUAL REPORT NUMBER 2, Serological diagnosis of gonorrhea, Contract N00014-74-A-0013-0002, Task No. NR 136-958, 31 August 1975.

5) Rudbach, J. A., M. K. Luoma, and E. C. B. Milner. STATUS REPORT No. 3, Serological diagnosis of gonorrhea, Contract N00014-76-C-0268, Task No. NR 204-004, 1 March 1976.

c) Index of publications.

Luoma, M. K. and J. A. Rudbach. A radioimmunoassay to detect antibody specific for Neisseria gonorrhoeae in human sera. A. S. M. Abstracts, pg. 40 (1975).

Luoma, M. K., W. R. Cross, and J. A. Rudbach. Radioimmunoassay for quantifying antibody to N. gonorrhoeae in human sera. Brit. J. Vener. Dis. 51: 387-391 (1975).

Milner, E. C. B. Characterization of an antigen of Neisseria gonorrhoeae. Master of Science Thesis, University of Montana (1976).

2 manuscripts are currently in preparation: one describes the modified RIA, which employs <sup>125</sup>I-labeled antigen for assaying antibodies to the

gonococcus; the second is a description of the physical, chemical, and biological characteristics of the gonococcal antigen.

d) Conclusions drawn from research data.

With a sensitive and specific radioimmunoassay technique it was possible to detect antibodies in the sera of 90% of males and females which have been infected with gonorrhea for more than 7 days or have recovered from a case of gonorrhea less than 6 months previously. The assay was on the verge of being modified and simplified to the point at which it would be feasible, economically and technically, to be put to use in clinical laboratories. The gonococcal specific antigen used in this assay was a low molecular weight glycoprotein without a highly folded tertiary structure.

e) List of major accomplishments.

- 1) An extract was prepared from virulent strains of Neisseria gonorrhoeae which contained a gonococcal specific antigen.
- 2) The gonococcal specific antigen was characterized as a small glycoprotein.
- 3) The gonococcal specific antigen was labeled, biosynthetically, with  $^{14}\text{C}$ .
- 4) A radioimmunoassay (RIA) was developed with this antigen, and this assay, with 90% accuracy, was capable of detecting antibodies stimulated during human gonococcal disease.
- 5) The gonococcal-specific antigen was successfully labeled, externally, with the easily quantifiable isotope,  $^{125}\text{I}$ .
- 6) The RIA was modified so that the gonococcal antigen labeled with  $^{125}\text{I}$  could be employed in the assay.

OFFICE OF NAVAL RESEARCH  
MICROBIOLOGY PROGRAM  
STANDARD DISTRIBUTION LIST

Number of copies:

- ( 12 ) Administrator, Defense Documentation Center  
Cameron Station  
Alexandria, VA 22314
- ( 6 ) Director, Naval Research Laboratory  
Attention: Technical Information Division  
Code 2027  
Washington, D.C. 20390
- ( 6 ) ~~Director, Naval Research Laboratory~~ Code 102JP (ONRL Doc)  
~~Attention: Library Code 2020 (ONRL)~~ Office of Naval Research  
~~Washington, D.C. 20390~~ 800 D. Quincy St.  
Arlington, VA 22217
- ( 3 ) Office of Naval Research  
Department of the Navy  
Code 443  
Arlington, Virginia 22217
- ( 2 ) Director, Research Division (Code 00)  
Naval Medical Research and Development Command  
National Naval Medical Center  
Bethesda, Maryland 20016
- ( 2 ) Technical Reference Library  
Naval Medical Research Institute  
National Naval Medical Center  
Bethesda, Maryland 20016
- ( 1 ) Office of Naval Research  
Department of the Navy  
Code 200  
Arlington, Virginia 22217
- ( 1 ) Office of Naval Research Branch Office  
495 Summer Street  
Boston, Massachusetts 02100
- ( 1 ) Office of Naval Research Branch Office  
536 South Clark Street  
Chicago, Illinois 60605

Enclosure 10.

OFFICE OF NAVAL RESEARCH  
MICROBIOLOGY PROGRAM  
STANDARD DISTRIBUTION LIST (Cont'd)

Number of copies:

- ( 1 ) Office of Naval Research Branch Office  
1030 East Green Street  
Pasadena, California 91101
- ( 1 ) Office of Naval Research  
Contract Administrator - Southeastern Area  
2110 G. Street, NW  
Washington, D.C. 20007
- ( 1 ) Commanding Officer  
U.S. Naval Medical Research Unit #2  
Box 14  
APO, San Francisco 96263
- ( 1 ) Commanding Officer  
U.S. Naval Medical Research Unit #3  
FPO, New York 09527
- ( 1 ) Commanding Officer  
U.S. Naval Medical Research Unit #5  
APO, New York 09319
- ( 1 ) Officer in Charge  
Submarine Medical Research Laboratory  
U.S. Naval Submarine Base, New London  
Groton, Connecticut 06342
- ( 1 ) Scientific Library  
U.S. Naval Medical Field Research Laboratory  
Camp Lejeune, North Carolina 28542
- ( 1 ) Scientific Library  
Naval Biosciences Laboratory  
Naval Supply Center  
Oakland, California 94625
- ( 1 ) Scientific Library  
Naval Aerospace Medical Research Institute  
Naval Aerospace Medical Center  
Pensacola, Florida 32512
- ( 1 ) Commanding Officer  
U.S. Naval Air Development Center  
ATTN: Aerospace Medical Research Department  
Johnsville, Warminster, PA 18974



OFFICE OF NAVAL RESEARCH  
MICROBIOLOGY PROGRAM  
STANDARD DISTRIBUTION LIST (Cont'd)

Number of copies:

- ( 1 )      Commanding General  
             U.S. Army Medical Research and  
             Development Command  
             Forrestal Building  
             Washington, D.C. 20314  
             Attn: MEDDH-SR
- ( 1 )      Director of Life Sciences  
             Air Force Office of Scientific Research  
             Bolling Air Force Base  
             Washington, D.C. 20032
- ( 1 )      STIC-22  
             4301 Suitland Road  
             Washington, D.C. 20390
- ( 1 )      Director  
             Walter Reed Army Institute of Research  
             Walter Reed Army Medical Center  
             Washington, D.C. 20012



REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER FINAL REPORT ✓	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) SEROLOGICAL DIAGNOSIS OF GONORRHEA.	5. TYPE OF REPORT & PERIOD COVERED Final Report. Sept. 1, 1973-30 Nov 1976	6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) Jon A. Rudbach	8. CONTRACT OR GRANT NUMBER(s) N00014-74-A-0013-0001-0002 N00014-76-C-0268	9. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS Task No. NR 136-958 and Task No. NR 204-004
9. PERFORMING ORGANIZATION NAME AND ADDRESS Department of Microbiology University of Montana Missoula, Montana 59801	10. CONTROLLING OFFICE NAME AND ADDRESS Procuring Contracting Officer, Office of Naval Research, Dept. of the Navy Arlington, VA 22217	11. REPORT DATE 1 Dec 1976
11. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office) Office of Naval Research, Resident Representative, University of Washington, 3710 Brooklyn Avenue, N. E., Unit No. 2 Seattle, Washington 98195	12. NUMBER OF PAGES 8	13. SECURITY CLASS. (of this report) NA
12. DISTRIBUTION STATEMENT (of this Report) This document has been approved for public release; its distribution is unlimited.	14. DECLASSIFICATION/DOWNGRADING SCHEDULE NA	
13. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report) same as above		
14. SUPPLEMENTARY NOTES		
15. KEY WORDS (Continue on reverse side if necessary and identify by block number) serodiagnosis of gonorrhea, radioimmunoassay for gonorrhea		
16. ABSTRACT (Continue on reverse side if necessary and identify by block number) An antigen specific for <u>Neisseria gonorrhoeae</u> was extracted with a surfactant from T-1 phase cells of strain F-62; this antigen was purified partially by fractional ethanolic precipitation and centrifugation. The antigen was characterized as a low molecular weight glycoprotein. With a <sup>14</sup> C-labeled preparation of this gonococcal antigen, a radioimmunoassay was developed for the serodiagnosis of gonorrhea. When 382 human sera from acutely infected and well documented negative control subjects were assayed by this procedure, 90% were diagnosed correctly, 5% were diagnosed incorrectly, and 5% of the		

DD FORM 1 JAN 73 1473

EDITION OF 1 NOV 65 IS OBSOLETE  
S/N 0102-014-6601

408843

Jace

~~20~~ results were indecisive. The assay procedure was modified in that the antigen was labeled with a more easily quantifiable isotope,  $^{125}\text{I}$ . In this latter system, 77% of the sera were diagnosed correctly, 13% were false positive or negative, and 10% of the results were indecisive. //